

### **REMARKS**

By the foregoing amendment, claims 1-3, 5, 6, 8, 10, 12 and 16 have been amended to further clarify their intended meaning. The claims have been amended to introduce clarifying that does not change the intended scope of the claims.

Claims 1 and 5 have been amended to introduce clarifying language. In particular, claims 1 and 5 have been amended to introduce the phrases “first polynucleotide comprising,” and “nucleophilic attack by a 5’-OH group of the acceptor polynucleotide on the polynucleotide-3’ phosphorothiolate” in order to clarify that the reaction includes nucleophilic attack of the acceptor 5’-OH group on the 3’ phosphorothiolate leaving group. Additionally, claims 6, 8, 12 and 16 have been amended to insert the phrase “nucleophilic attack by a 5’-OH group of the acceptor polynucleotide on the polynucleotide-3’ phosphorothiolate” in order to clarify that the reaction includes nucleophilic attack of the acceptor 5’-OH group on the 3’ phosphorothiolate leaving group to produce a phosphodiester. Claim 6 has been amended to add the term “intermediate” for the same reason that “first” was included in claims 1 and 5. Claims 5, 6 and 16 have been amended as discussed below under the discussion of the § 112, second paragraph, rejection.

The foregoing amendments are supported by the originally filed specification. In particular, addition of the phrase relating to nucleophilic attack is supported by, for example, the paragraph at the top of page 27 as well as the paragraph spanning pages 32 and 33 of the specification. The phrases relating to a “first polynucleotide” and “intermediate polynucleotide” are added merely as clarifying language, in order to distinguish the polynucleotide that originally has a polynucleotide 3’ phosphorothiolate from the acceptor polynucleotide, and to clarify that the 3’ phosphorothiolate is displaced by the nucleophilic attack by the 5’-OH group of the

acceptor polynucleotide. This language parallels that of claim 8, wherein a vector is recited as comprising a polynucleotide 3' phosphorothiolate. The other clarifying amendments are supported by the originally filed claims and the specification.

No new matter has been added by the foregoing amendments.

The pending claims are 1-16.

Response to Specific Issues Raised by the Office Action

Claims 5-7 and 16 were rejected under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. In particular, claim 5 was objected to for reciting "said polynucleotide product," for which there was allegedly no antecedent basis in the claims. Applicants have addressed this rejection by amending claim 5 to replace "said polynucleotide" with "said ligated nucleic acid," which has specific antecedent basis in the claim. Applicants submit that this amendment has overcome the rejection.

Additionally, claims 6 and 16 were rejected for reciting "said iodonitrobenzene," which allegedly lacked antecedent basis. Applicants have obviated this rejection by replacing the term "iodonitrobenzene" in claims 6 and 16 with the term "activator," which finds antecedent within claims 6 and 16, respectively. Thus, Applicants submit that the amendment to claims 6 and 16 have overcome the rejection.

Claim 7 was apparently included in the § 112, second paragraph, rejection because it depends from claim 6. Applicants submit that the rejection of claim 7 has been overcome by the amendment to claim 6 for the same reasons as stated above.

For the foregoing reasons, Applicants submit that the rejection of claims 5-7 and 16 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Claim 1 was rejected under 35 U.S.C. § 102(b) as being anticipated by Letsinger et al. (US Patent No. 5,476,930; hereinafter "Letsinger"). Applicants respectfully traverse this rejection.

In order for a reference to anticipate a claim, the reference must teach each and every element of the claim. While such teaching may be explicit or inherent, in any case the USPTO bears the initial burden of propounding a *prima facie* case of anticipation, in which it must set forth how the reference meets each claim element. In the present case, Letsinger fails to provide teaching of each element of claim 1. Thus, the rejection under § 102(b) is improper and should be withdrawn.

Claim 1 provides:

A method of non-enzymatic ligation of a nucleic acid, comprising contacting a first polynucleotide comprising a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide under conditions that allow nucleophilic attack by a 5'-OH group of the acceptor polynucleotide on the polynucleotide-3' phosphorothiolate to form a phosphodiester bond between said first polynucleotide and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said first polynucleotide and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated. [Emphasis added].

In setting forth its reasons for the rejection, the Office Action states:

[S]ince Letsinger *et al.* teach a method of forming an oligonucleotide by: a) disposing at least two oligonucleotides in aqueous solution wherein one of the oligonucleotides has a 5' terminal bromoacetyl amino group and the other of the nucleotide has a 3' terminal **phosphorothioate** group; and b) covalently binding the oligonucleotides together through the  **$\alpha$ -haloacyl** group and the

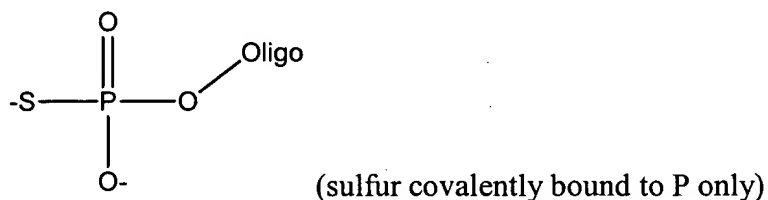
**phosphothioate** groups spontaneously forming a **thiophosphorylacetylamino** group therebetween (see columns 9 and 10, claims 1-7), Letsinger *et al.*, disclose contacting a polynucleotide-3' **phosphorothiolate** and said acceptor polynucleotide, wherein a **phosphodiester** bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated as recited in claim 1.

Therefore, Letsinger *et al.*, teach all limitations recited in claim 1. [Emphasis added by Applicants].

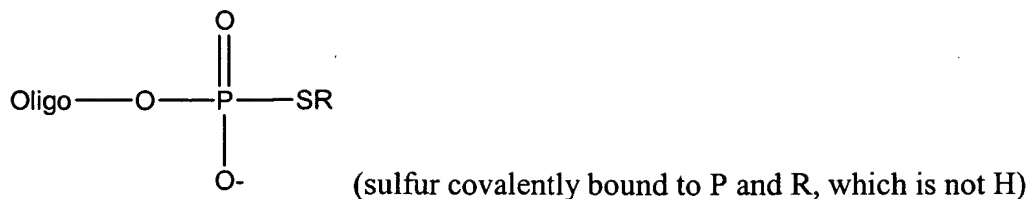
Applicants submit that the quoted passage from the Office Action itself demonstrates that the reference fails to teach the present invention. As accurately described by the Office Action, Letsinger teaches combining an oligonucleotide having a 5'-terminal bromoacetylamino group with another oligonucleotide having a 3' terminal **phosphorothioate** group. The two oligonucleotides covalently bind with one another through the  $\alpha$ -haloacyl group and the **phosphorothioate** group to form a **thiophosphorylacetylamino** group between the two oligonucleotides. This reaction is depicted in Scheme A in the attached Appendix.

In contrast, the present invention entails contacting a polynucleotide-3' **phosphorothiolate** and an acceptor polynucleotide, wherein a **phosphodiester** bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide. This reaction is depicted in Scheme B in the attached Appendix.

The Letsinger reaction of a phosphorothioate:

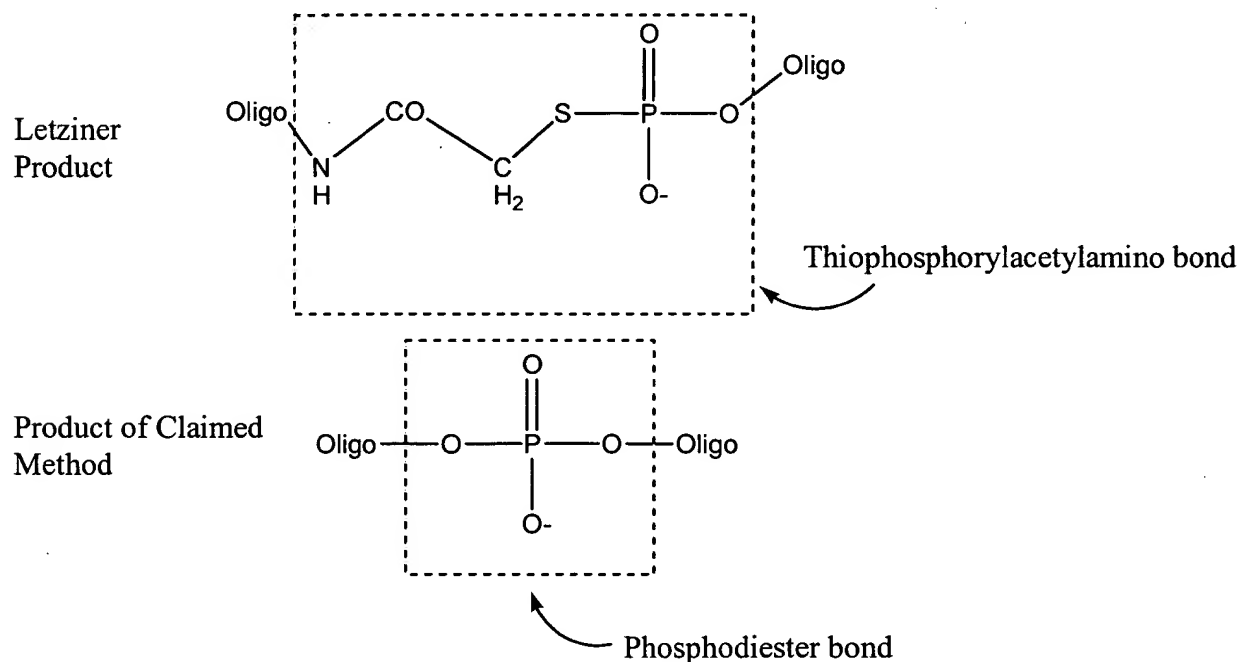


with a bromoacetylamino group yields an entirely different product from the claimed process, wherein a phosphorothiolate:



is reacted with a free hydroxyl group of the acceptor polynucleotide. Note that in the phosphorothioate (Letsinger) the sulfur group is free in solution and acts as a nucleophile, not a leaving group, whereas the phosphorothiolate (instant claims) is covalently bonded to a moiety R, thereby rendering it an unsuitable nucleophile, but a suitable leaving group.

For the convenience of the PTO, the products of the Letsinger process and the claimed method are compared immediately below:



As can be seen in the foregoing comparison, the product of the Letsinger process is a completely different chemical structure from that produced by the claimed process. The Letsinger process yields a thiophosphorylacetylaminic bond (top dashed box) between the two oligonucleotides (oligo), whereas the claimed process yields a phosphodiester bond (bottom

dashed box) between the two oligonucleotides. Thus Letsinger fails to teach the recited phosphodiester of claim 1.

Letsinger also fails to teach the recited phosphorothiolate of claim 1. (Hereafter underscoring will be used to emphasize the difference in spelling between phosphorothiolates and phosphorothioates. The difference in spelling has a profound impact on the meaning of the words. Phosphorothiolate indicates that the sulfur is covalently bonded to both P and a second, carbon-containing moiety, whereas phosphorothioate indicates that the sulfur is covalently bonded to P only, the other valency being satisfied by an acidic H.) Applicants submit that, as Letsinger discloses reaction of a phosphorothioate (good nucleophile, poor leaving group) with an acylhalide, Letsinger fails to teach or fairly suggest reaction of a phosphorothiolate (poor nucleophile; good leaving group) with a free hydroxyl group to form a phosphodiester bond. Thus, Letsinger fails to teach a necessary element of claim 1, i.e. reaction of a phosphorothiolate, and thus fails to anticipate the claim.

For the foregoing reasons, Applicants submit that the rejection of claim 1 under 35 U.S.C. § 102(b) as being anticipated by Letsinger is untenable and should be withdrawn.

The Office Action also includes a rejection of claims 1, 3 and 4 under 35 U.S.C. § 102(b) as being anticipated by Albrecht *et al.* (US Patent No. 6,013,445; hereinafter "Albrecht"). As Albrecht fails to teach at least one limitation of claim 1, and by extension of claims 3 and 4, Applicants respectfully submit that this rejection is untenable and should be withdrawn.

The text of claim 1 is recited above. Claim 3, which is directly dependent from claim 1, is further limited in that the first polynucleotide further comprises a duplex polynucleotide. Claim 4, which is dependent from claim 1, further provides that the acceptor polynucleotide

further comprises a duplex polynucleotide. Applicants submit that Albrecht fails to teach at least one limitation of claim 1, and thus fails to anticipate claim 1 as well as dependent claims 3 and 4.

The Office Action states:

Regarding claims 1, 3, and 4, since Albrecht *et al.*, teach that ligating (120) an encoded adaptor (having duplex polynucleotide) to an end of the polynucleotide (122)(having duplex polynucleotide), the end of the polynucleotide having a dephosphorylated 5' hydroxyl and the end of the encoded adaptor (124) to be ligated having a first strand (126) and a second strand (128), the second strand of the encoded adaptor having a 3' blocking group (130); (b) removing the 3' blocking group of the second strand after ligation by chemically removing the group in situ; (c) phosphorylating (134) the 5' hydroxyl of the polynucleotide; d) ligating (136) a second strand (142) having an unblocked 3' moiety to regenerate the encoded adaptor (138); and (e) identifying (144) one or more nucleotides at the end of the polynucleotide by identity of the encoded adaptor ligated thereto, e.g. via a fluorescently labeled (140) tag complement (see Figure 3A and column 21, second paragraph) wherein 3' blocking group is phosphorothiolate (see column 19, second paragraph), Albrecht *et al.*, disclose contacting a polynucleotide-3' phosphorothiolate with an acceptor polynucleotide (5' hydroxyl) under conditions that allow formation of a phosphodiester bond between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, wherein a phosphodiester bond is formed between said polynucleotide-3' phosphorothiolate and said acceptor polynucleotide, whereby a ligated nucleic acid product is generated as recited in claim 1 wherein said polynucleotide-3' phosphorothiolate further comprises a duplex polynucleotide as recited in claim 3 and said acceptor polynucleotide further comprises a duplex polynucleotide as recited in claim 4.

Therefore, Albrecht *et al.*, teach all limitations recited in claims 1, 3, and 4.

Applicants submit that Albrecht fails to teach or fairly suggest at least two elements of claims 1, 3 and 4. In particular, Applicants note that the present claims require reaction between a 5'-hydroxyl group of an acceptor polynucleotide and a 3' phosphorothiolate of a first polynucleotide having a polynucleotide-3' phosphorothiolate group. In contrast, and contrary to what is stated in the Office Action, Albrecht fails to teach or even suggest 3' phosphorothiolate moieties. Although the Office Action points to column 19, second paragraph, as teaching phosphorothiolate, no such teaching is found in that paragraph (or anywhere else in the patent

disclosure). Instead, this paragraph teaches phosphorothioate, as is evident from the following excerpt from the patent disclosure:

The 3' blocking group "z" may have a variety of forms and may include almost any chemical entity that precludes ligation and that does not interfere with other steps of the method, e.g. removal of the 3' blocked strand, ligation, or the like. Exemplary 3' blocking groups include, but are not limited to, hydrogen (i.e. 3' deoxy), phosphate, phosphorothioate, acetyl, and the like. Preferably, the 3' blocking group is a phosphate because of the convenience in adding the group during the synthesis of the 3' blocked strand and the convenience in removing the group with a phosphatase to render the strand capable of ligation with a ligase. An oligonucleotide having a 3' phosphate may be synthesized using the protocol described in chapter 12 of Eckstein, Editor, Oligonucleotides and Analogues: A Practical Approach (IRL Press, Oxford, 1991). [Emphasis added].

As pointed out above, the difference between a phosphorothioate (Albrecht) and a phosphorothiolate is notable. The person skilled in the art would recognize that a phosphorothioate has a sulfur that is normally ionized in solution and is therefore suitable as a nucleophile. In stark contrast, the person skilled in the art would recognize that the claimed 3' phosphorothiolate would not be a good nucleophile. In other words, the phosphorothioate taught by Albrecht is too chemically dissimilar from the phosphorothiolate of the present claims for the former to anticipate the latter.

This chemical difference between phosphorothioates and phosphorothiolates also gives rise to a second difference between Albrecht and the present claims. In the Albrecht process, the blocking group "z" must first be removed before the two polynucleotide strands can be ligated. In the case where the "z" group is a phosphate, it is removed by a phosphatase. (It is not clear from the Albrecht disclosure how the "z" group would be removed if it were a phosphorothioate, however it must be removed.) In contrast, the 3' phosphorothiolate of the present invention is not a blocking group that needs to be removed, but a leaving group that is displaced by the 5'-hydroxyl group of the adaptor polynucleotide. The person of skill in the art would simply not accept that the process described by Albrecht, which includes using a 3' phosphorothioate

blocking group, would anticipate the claimed process, which includes nucleophilic attack of a 5'-hydroxyl group on a 3' phosphorothioate leaving group.

Applicants respectfully submit that the foregoing analysis shows that Albrecht fails to anticipate claim 1, and by extension, claims 3 and 4. Withdrawal of the rejection is therefore requested.

The Office Action also includes a rejection of claims 8, 12 and 15 as being obvious over Albrecht and Robbins *et al.*, (U.S. Patent No. 5,843,648; hereinafter "Robbins"). Applicants submit that this rejection is untenable and should be withdrawn.

In order to render a claim obvious, a combination of references must teach or fairly suggest each limitation of the claim. The initial burden is on the Patent Office to establish a *prima facie* case of obviousness by a preponderance of the evidence. Applicants submit that the combination of Albrecht and Robbins fails to teach each element of the claims. Thus, the Office Action fails to establish a *prima facie* case of obviousness against claims 8, 12 and 15.

Although the Office Action purports to undertake a detailed analysis of the claims and how the references allegedly meet all the limitations thereof, the rejection is premised on the same confusion between phosphorothioate and phosphorothiolate as has been discussed at length above. In short, the Office Action states, erroneously, that Robbins teaches a vector having a phosphorothiolate at column 31, lines 39-50. This is incorrect, because Robbins teaches a phosphorothioate, not phosphorothiolate. In the passage referred to by the Office Action, Robbins states:

The use of COS cells transfected with a series of truncated cDNA generated by the exonuclease III gene deletion method was used to locate region containing an immunogenic epitope of tyrosinase. In order to create unidirectional deletions, the vector was cut with Xba I and filled with .alpha.-phosphorothioate deoxynucleotides which protect the plasmid from digestion with exonuclease III.

The vector was also digested with Not I, which served as the starting point for digestion. Since the deletion can be controlled by varying the time of digestion, various sizes of the truncated gene can be generated by this method and the region containing the epitopes can be narrowed. [Emphasis added].

As has been discussed at length above, phosphorothioates are chemically distinct from phosphorothiolates. In addition to the aforementioned differences in nucleophilic properties, Robbins teaches phosphorothioates as nuclease-resistant analogs of phosphodiester in the manufacture of vectors. However, Robbins nowhere even mentions phosphorothiolates. Nor does Robbins teach or fairly suggest that phosphorothiolates would make suitable substitutes for the phosphorothioates explicitly taught by Albrecht and Robbins. Accordingly, Applicants submit that Robbins fails to supply the critical teaching of a phosphorothiolate that is missing from Albrecht, and which is a necessary element of each of claims 8, 12 and 15. Thus the combination of Albrecht and Robbins fails to establish a *prima facie* case of obviousness against claims 8, 12 and 15. This ground of rejection should therefore be withdrawn.

The Office Action also contains a rejection of claims 5, 9 and 13 under 35 U.S.C. § 103(a) as being obvious over Albrecht, Robbins and Panayotatos (U.S. Patent No. 5,256,568; hereinafter "Panayotatos"). Applicants respectfully submit that this rejection is untenable and should be withdrawn.

As discussed in detail above, neither Albrecht nor Robbins teaches or fairly suggests use of a 3' phosphorothiolate. A careful review of Panayotatos, which is directed to vectors and transformed mast cells, also fails to teach or fairly suggest 3' phosphorothiolate. Nor is there any suggestion within Panayotatos that would cause the person skilled in the art to substitute the claimed phosphorothiolate for the phosphorothioate disclosed by Albrecht and Robbins. Thus, the combination of Albrecht, Robbins and Panayotatos fails to establish the requisite *prima facie* case of obviousness.

For the foregoing reasons, Applicants submit that the rejection of claims 5, 9 and 13 under 35 U.S.C. § 103(a) over the combination of Albrecht, Robbins and Panayotatos is untenable and should be withdrawn.

Applicants gratefully acknowledge the indication of allowable subject matter.


### **CONCLUSION**

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. Should the Examiner have any questions or suggestions for expediting prosecution, he is invited to contact the undersigned.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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**Date: November 16, 2004**